OIPE
JAN 0 6 2005
PATENT & TRADEMARK OFFICE

Copy

09/991458

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on the date shown below:

January 3, 2005

David Saliwanchik

David R. Saliwanchik, Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. UF-283
Patent No. 6,766,613

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Bruce R. Stevens, James P. Cuda, Lewis S. Long
Issued	:	July 27, 2004
Patent No.	:	6,766,613
For	:	Materials and Methods for Controlling Pests

Certificate
JAN 12 2005
of Correction

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 2, line 61:	Page 3, line 25 (substitute specification):
"bunyarnidera The"	-- bunyarnidera. The --

Patent Reads:	**Application Reads:**
Column 5, line 45:	Page 7, line 24 (substitute specification):
"(< 0.001"	--(≤0.001--
Column 10, line 15:	Page 14, line 15 (substitute specification):
"276(36):3341.3-33418"	--276(36):33413-33418--
Column 11, line 42:	Page 16, line 16 (substitute specification):
"CDNA"	--cDNA--
Column 15, line 50:	Page 22, line 29 (substitute specification):
"15-224-229"	--15:224-229--
Column 19, line 56:	Page 29, line 7 (substitute specification):
"(GGLAA"	--(GGIAA --
Column 20, line 56:	Page 30, line 19 (substitute specification):
"Nemstian"	--Nernstian--
Column 20, line 57:	Page 30, line 19 (substitute specification):
"activity, (c) amino"	--activity, (e) amino--

A true and correct copy of pages 3, 6, 7, 9, 14, 16, 22, 29 and 30 of the substitute specification as filed on December 24, 2003, which support Applicants' assertion of errors on the part of the Patent Office, as well as a corrected Official Filing Receipt in which inventor Cuda's name was corrected, accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

David Saliwanchik

David R. Saliwanchik
Patent Attorney
Registration No. 31,794
Phone: 352-375-8100
Fax No.: 352-372-5800
Address: P.O Box 142950
Gainesville, FL 32614-2950

DRS/la

Attachments: Certificate of Correction in duplicate;
Corrected Filing Receipt; and
True copies of pages 3, 6, 7, 9, 14, 16, 22, 29 and 30 as filed in a substitute specification dated December 24, 2003

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,766,613

DATED : July 27, 2004

INVENTORS : Bruce R. Stevens, James P. Cuda, Lewis S. Long

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page, Inventors,
"James S. Cuda" should read -- James P. Cuda --.

Column 2,
Line 61, "bunyarnidera The" should read --bunyarnidera. The --.

Column 4,
Line 52, "Na^{+}, Ca^{-}-coupled" should read -- Na^{+}-, Ca^{-}-coupled --.

Column 5,
Line 45, "(< 0.001" should read --($\leq$0.001--.

Column 10,
Line 15, "276(36):3341.3" should read --276(36):33413--.

Column 11,
Line 42, "CDNA" should read --cDNA--.

Column 15,
Line 50, "15-224-229" should read --15:224-229--.

Column 19,
Line 56, "(GGLAA" should read --(GGIAA--.

MAILING ADDRESS OF SENDER:

Saliwanchik, Lloyd & Saliwanchik
P.O Box 142950
Gainesville, FL 32614-2950

PATENT NO. 6,766,613
No. of add'l. copies
@ 30¢ per page

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,766,613

DATED : July 27, 2004

INVENTORS : Bruce R. Stevens, James P. Cuda, Lewis S. Long

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 20,
Line 56, "Nemstian" should read -- Nernstian --.

Column 20,
Line 57, "activity, (c) amino" should read –activity, (e) amino--.

MAILING ADDRESS OF SENDER:

Saliwanchik, Lloyd & Saliwanchik
P.O Box 142950
Gainesville, FL 32614-2950

PATENT NO. 6,709,820
No. of add'l. copies
@ 30¢ per page

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,766,613

DATED : July 27, 2004

INVENTORS : Bruce R. Stevens, James P. Cuda, Lewis S. Long

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page, Inventors,
"James S. Cuda" should read -- James P. Cuda --.

Column 2,
Line 61, "bunyarnidera The" should read --bunyarnidera. The --.

Column 4,
Line 52, "Na^+, Ca^--coupled" should read -- Na^+-, Ca^--coupled --.

Column 5,
Line 45, "(< 0.001" should read --($\leq$0.001--.

Column 10,
Line 15, "276(36):3341.3" should read --276(36):33413--.

Column 11,
Line 42, "CDNA" should read --cDNA--.

Column 15,
Line 50, "15-224-229" should read --15:224-229--.

Column 19,
Line 56, "(GGLAA" should read --(GGIAA--.

MAILING ADDRESS OF SENDER:

Saliwanchik, Lloyd & Saliwanchik
P.O Box 142950
Gainesville, FL 32614-2950

PATENT NO. 6,766,613
No. of add'l. copies
@ 30¢ per page

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,766,613

DATED : July 27, 2004

INVENTORS : Bruce R. Stevens, James P. Cuda, Lewis S. Long

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 20,
Line 56, "Nemstian" should read -- Nernstian --.

Column 20,
Line 57, "activity, (c) amino" should read –activity, (e) amino--.

MAILING ADDRESS OF SENDER:

Saliwanchik, Lloyd & Saliwanchik
P.O Box 142950
Gainesville, FL 32614-2950

PATENT NO. 6,709,820
No. of add'l. copies
@ 30¢ per page

OIPE JC#12 JAN 06 2005 PATENT & TRADEMARK OFFICE

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
Washington, DC 20231
www.uspto.gov

APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/991,458	11/16/2001	1638	690	UF-283	4	39	5

CONFIRMATION NO. 3818

CORRECTED FILING RECEIPT

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
2421 N.W. 41ST STREET
SUITE A-1
GAINESVILLE, FL 326066669

RECEIVED DEC 23 2002

10C000000009252322*

Date Mailed: 12/17/2002

Receipt is acknowledged of this regular Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Filing Receipt Corrections, facsimile number 703-746-9195. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Bruce Russell Stevens, Gainesville, FL;
James P. Cuda, Gainesville, FL;
Lewis S. Long, Gainesville, FL;

Domestic Priority data as claimed by applicant

Foreign Applications

Projected Publication Date: To Be Determined - pending completion of Security Review

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Materials and methods for controlling pests

Preliminary Class

The most widely used environmentally friendly pesticidal formulations developed in recent years have been microbial pesticides derived from the bacterium *Bacillus thuringiensis ("B.t.")*. *B. thuringiensis* is a Gram-positive bacterium that produces proteins which are toxic to certain orders and species of pests. Many different strains of *B. thuringiensis* have been shown to produce insecticidal proteins. Compositions including *B. thuringiensis* strains which produce insecticidal proteins have been commercially-available and used as environmentally-acceptable insecticides because they are toxic to specific target pests, but are harmless to plants and other non-target organisms. The specificity of these toxins is often strain-specific, with certain toxins being active against a relatively narrow spectrum of pests. Indeed, many *B.t.* toxins have been identified that are active only against particular insect orders (*e.g.*, dipterans, hymenopterans, coleopterans, etc.). This limitation prevents the use of a single *B.t.* endotoxin composition as a broad-range pesticide.

Crop pests are not the only targets for which an environmentally friendly and safe pesticide would be highly desirable. Many blood-feeding pests are known to prey on humans and animals, and many pests are vectors for pathogenic microorganisms that threaten human and animal health, including commercially important livestock, pets and other animals. The order Diptera contains a large number of blood-ingesting and disease-carrying pests, including, for example, mosquitoes, black flies, no-see-ums (punkies), horse flies, deer flies and tsetse flies. Various species of mosquitoes, for example, transmit diseases caused by viruses, and many are vectors for disease-causing nematodes and protozoa. Mosquitoes of the genus *Anopheles* transmit *Plasmodium*, the protozoan that causes malaria. The mosquito species *Aedes aegypti* transmits an arbovirus that causes yellow fever in humans. Other viruses transmitted by *Aedes* species include the causative agents of dengue fever, eastern and western encephalitis, Venezuelan equine encephalitis, St. Louis encephalitis, chikungunya, oroponehe and bunyarnidera. The genus *Culex*, which includes the common house mosquito *C. pipiens*, is implicated in the transmission of various forms of encephalitis, filarial worms, and West Nile virus. *Trypanasoma cruzi*, the causative agent of Chagas disease, is transmitted by various species of blood ingesting Triatominae bugs. Tsetse flies (*Glossina spp.*) transmit African trypanosomal diseases of humans and cattle. Other diseases are transmitted by various blood-ingesting pest species.

~250 mV (Harvey *et al.*, 1999, *Am. Zool.* 38:426-441; Harvey and Wieczorek, 1997, *J. Exper. Biol.* 200:203-216). Epithelial cells of this region transport a variety of nutrients, including nutrient amino acids and electrolytes, as demonstrated by i*n vitro* isolated membrane vesicle uptake studies. In place of a Na^+/K^+-ATPase typically found in cells, this tissue instead possesses a proton translocating V-ATPase (Graf *et al.*, 1992, *FEBS Lett.* 300:119-122; Merzendorfer *et al.*, 1997, *J. Exper. Biol.* 200:225-235) which energizes the cell membranes for secretion and absorption of K^+ and Na^+ ions, and establishment of a large pH gradient. A K^+-activated leucine-preferring transporter (KAAT1) has been identified from the hornworm midgut (Castagna *et al.*, 1998, *Proc Natl. Acad Sci. USA* 95:5395-5400), and a GABA (gamma aminobutyric acid) transporter has been cloned from an *M. sexta* embryo cDNA library (Mbungu *et al.*, 1995, *Arch. Biochem. Biophys.* 318:489-497).

CAATCH1 (**C**ation-**A**mino **A**cid **T**ransporter/**CH**annel) is a recently cloned insect membrane protein initially cloned from *Manduca sexta*; CAATCH1 exhibiting a unique polypeptide and nucleotide sequence related to, but different from, mammalian Na^+-, Cl^--coupled neurotransmitter transporters (Feldman *et al.*, 2000, *J. Biol. Chem.* 275:24518–24526). Utilizing a unique PCR-based strategy, the gene encoding CAATCH1 was cloned (Feldman *et al.*, 2000, *supra*) from a cDNA library in LambdaZap plasmids, obtained from the digestive midgut of *Manduca sexta* larvae.

The unanticipated and novel biochemical, physiological, and molecular properties of CAATCH1 indicated that it is a multi-function protein that mediates amino acid uptake in a manner that is thermodynamically uncoupled from ion electrochemical potentials, and furthermore that CAATCH1 simultaneously functions as an amino acid-modulated gated alkali cation channel (Quick, M., and B.R. Stevens, 2001, "Amino acid transporter CAATCH1 is also an amino acid-gated cation channel". *J. Biol. Chem* 276: 33413-33418) serving at least Na^+ and K^+. Radiotracer and electrophysiology experiments with functional CAATCH1 polypeptide expressed from the full length CAATCH1 cDNA demonstrated direct amino acid ligand-protein interactions, and indicated that binding by different amino acid substrates differentially affects the conformational states of CAATCH1 (Quick, M. and B.R. Stevens, 2001, *J. Biol. Chem.* 276:33413-33418). Notably, L-methionine binding to CAATCH1 *in situ* in biomembranes in the presence of Na^+ perturbs the charge-voltage relation with a high affinity binding constant,

affecting transient currents due to CAATCH1-associated charge transfer across the membrane dielectric field. Furthermore, CAATCH1-associated voltage-dependent amino acid-elicited steady state inward cation currents are blocked by methionine, and indeed methionine reversed charge movements via CAATCH1 expressed in cell membranes, even though radiotracer methionine influx is catalyzed by CAATCH1 (Quick, M. and B.R. Stevens, 2001, *J. Biol. Chem.* 276:33413-33418). In insects, CAATCH1 likely plays a broader role than that of a 'classical' transporter or channel; as a multifunction protein CAATCH1 is likely a key protein in electrolyte and organic solute homeostasis of certain insects (Feldman *et al*, 2000, *J. Biol. Chem.* 275(32):24518-24526)

Many pests—including mosquitoes, Lepidopterans, and Coleopterans—possess an alkaline pH midgut, and share some similar physiological mechanisms that occur within this unusual milieu (Nation, J., 2001, *In*: Insect Physiology and Biochemistry, CRC Press, Boca Raton, pp 496). Mosquito larvae possess such an alkaline midgut, and adjust free amino acid concentrations in their hemolymph and extracellular compartments in direct response to existence of foreign factors in the gut (e.g., *B.t.* δ–endotoxin) or the salinity of their habitat (Bounias, M. *et al.,* 1989, *J. Invertebr Pathol.* 54:16-22). Notably, one standout free amino acid, L-proline, accumulates 4-fold during the normal course of *Aedes aegypti* larval development, and in *Culex spp.* L-proline accumulation can exceed 50-fold (up to 70 mM) when larvae are stimulated by Na^+ in their feeding pools (Bounias, M. *et al.,* 1989, *supra*; Patrick, M.L. and T.J. Bradley, 2000, *J. Exp. Biol.* 203:831-839; Chaput, R.L. and J.N. Liles, 1969, *Ann. Entomol. Soc. Am.* 62:742-747). This proline is likely utilized as an energy source and for osmoregulation (Patrick, M.L. and T.J. Bradley, 2000, *J. Exp. Biol.* 203:831-839; Bounias *et al.,* 1989, J. Invertebr Pathol. 54:16-22). In contrast, free L-methionine has the distinction of virtually the lowest measurable concentration ($\leq$ 0.001 mM) of any of the free amino acids in mosquito larvae. Virtually all methionine existing in the free amino acid state in larvae (Dadd, R.H., 1973, *Ann Rev Entomol* 18:381-420) is metabolically shunted and sequestered into so-called methionine-rich hexamerin proteins (Korochkina *et al.*, 1997, *Insect Biochem Mol Biol.* 27:813-824) that are stored for post-larval developmental events. In its role as a nutrient transporter, the CAATCH1 protein has been shown to be primarily responsible for proline uptake (Feldman *et al.*, 2000, *J. Biol. Chem.* 275(32):24518-24526), while in the presence of extremely low

proteins, can be administered to a pest, or its situs, to achieve pest control. Specifically exemplified herein is the use of the amino acids methionine or leucine (and/or analogs thereof) to control pests. These compounds can be administered in a wide range of ways including the application of compositions comprising these individual amino acids (and/or their analogs, or bound to other molecules, for example by amide bonds), application of polypeptides which are made up of an abundance of these pesticidal amino acids, and providing a transgenic plant which expresses a pesticidal amount of the amino acids as either free amino acids, as salts of amino acids or their analogs, or bound to other molecules, or existing in polypeptides.

In a preferred embodiment, the methods of the subject invention involve delivering to or applying to a pest a composition that comprises methionine or leucine, or an analog thereof. Exemplary analogs include, but are not limited to, methionine esters, leucine esters, D-methionine, D-leucine, D-tert-leucine, L-tert-leucine, DL-methionine, DL-leucine, L-methioninol, L-leucininol, L- or D-methioninemethylsulfonaium chloride, small methionyl peptides, low molecular weight leucyl peptides, alphaketoisocaproic acid, and the like. Likewise, methyl or ethyl esters of such compounds are also contemplated to be useful, as are keto derivatives of such compounds. In a specific embodiment, insect pests are controlled by administering methionine to the insects. Alternatively, other amino acids such as histidine, glycine, threonine, or alanine can also be used. For all embodiments involving compounds with chiral centers, the L-, D-, DL-, or partially racemic forms are also contemplated to be useful. Alternatively, amino acid analogs that do not possess chiral centers are contemplated to be useful.

Additional target crops to be protected within the scope of the present invention comprise, e.g., the following species of plants:

Although it is believed that the administration of amino acids to achieve pest control according to the subject invention is effective as a result of the disruption of particular proteins as described herein, one aspect of the subject invention is simply the control of pests by administering amino acids or these analogs (regardless of the specific mechanism involved).

One aspect of this invention contemplates altering methionine levels in pests by manipulating biochemical pathways leading to methionine production in target pests. Such

to CAATCH1 and blocking cation current *via* the ion channel aspect of the CAATCH1 polypeptide. By interfering with the combined amino acid transporting/ligand-gated K^+/Na^+ ion channel properties of CAATCH1, the homeostasis-maintaining role of the protein is perturbed and leads to disruption of fluid, electrolyte and nutrient movements.

Although the disruption of the function of the CAATCH1 protein from *M. sexta*, *A. aegypti*, or *Leptinotarsa decemlineata* is specifically exemplified herein, the materials and methods of the subject invention encompass the analogous disruption of other related transporters, channels, or multifuncion transporter/channel proteins in other target pests. Further, the subject invention contemplates direct inhibition of the CAATCH1 class of proteins as well as the disruption of the cascade of cellular activities associated with these unique transporter proteins. In its role as a nutrient transporter, the CAATCH1 protein has been shown to be primarily responsible for proline uptake (Feldman *et al.*, 2000, *supra*), while in the presence of extremely low concentrations of methionine, CAATCH1 effectively shuts down ionic fluxes via its channel aspect (Feldman *et al.*, 2000, *J. Biol. Chem.* 275(32)24518-24526; Quick, M. and B.R. Stevens, 2001, *J. Biol. Chem.* 276(36):33413-33418).

As would be appreciated by one skilled in the art having the benefit of the instant disclosure, pest control can be achieved in a number of ways. For example, compositions comprising individual pesticidal amino acids can be administered directly to the pests. Alternatively, these amino acids may be provided as components of polypeptides, other covalently linked polymers of amino acids or their analogs, or salts of amino acids or their analogs, which are then degraded to the individual amino acids or their analogs in the pest gut and/or prior to entry into the pest gut. These polypeptides or covalently linked compounds may be produced synthetically or recombinantly. In the case of recombinant production, the polypeptides may be produced, for example, in a microbial host followed by isolation of the polypeptide for subsequent administration. The microbial produced polypeptide may also be administered directly to the pest and/or its *situs*.

The polypeptides which contain the pesticidal amino acids of the subject invention may also be expressed in plant cells. The polypeptide may be isolated from the plant cell or, preferably, pest control is achieved by a pest ingesting the plant material. Furthermore pest

The word "transform" is broadly used herein to refer to introduction of an exogenous polynucleotide sequence into a prokaryotic or eukaryotic cell by any means known in the art (including for example, direct transmission of a polynucleotide sequence from a cell or virus particle, transmission by infective virus particles and transmission by any other nucleotide-bearing construct) resulting in a permanent or temporary alteration of genotype.

The term "upstream" refers to DNA adjacent to the 5' portion of a DNA sequence; whereas, the term "downstream" refers to DNA adjacent to the 3' portion of a DNA sequence.

A "transgenic cell" is any cell derived or regenerated from a transformed cell or derived from a transgenic cell. Exemplary transgenic cells include plant calli derived from a transformed plant cell and particular cells such as leaf, root, stem, *e.g.*, somatic cells, or reproductive (germ) cells obtained from a transgenic plant.

As used herein, a "transgenic plant" is a plant or progeny thereof derived from a transformed plant cell or protoplast, wherein the plant DNA contains an introduced exogenous DNA molecule not originally present in a native, non-transgenic plant of the same strain.

In accordance with the present invention, nucleic acid sequences include and are not limited to, DNA, including and not limited to cDNA and genomic DNA, and genes; RNA, including and not limited to mRNA and tRNA; antisense sequences; and recombinant vectors, including, for example, plasmids, cosmids, phagemids, artificial chromosomes, phage, viruses, baculoviruses, and the like.

The term "gene" is used for simplicity to refer to a functional protein-, polypeptide- or peptide-encoding unit. As will be understood by those in the art, this functional term includes genomic sequences, operon sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides or peptides.

As used herein with regard to molecular biology, a "vector" is a DNA molecule capable of replication in a host cell and/or to which another DNA segment can be operatively linked so as to bring about replication of the attached segment. A plasmid is an exemplary vector. Note, however, that in entomology terms, a "vector" is a disease transmitting insect.

The terms "polypeptide", "peptide", and "protein" as used herein refer to amino acid sequences of two or more amino acids.

linkages are also provided. Derivatives of the foregoing fusion proteins are also provided (*e.g.*, branched, cyclized, or C-terminal chemically modified, etc.).

Virtually any polypeptide-encoding DNA sequence may be fused to the sequences disclosed herein in order to encode a fusion protein. This includes DNA sequences that encode targeting peptides, proteins for recombinant expression, proteins to which one or more targeting peptides are attached, protein subunits, and the like. Such modifications to primary nucleotide sequences to enhance, target, or optimize expression of the gene sequence in a particular host cell, tissue, or cellular localization, are well known to those of skill in the art of protein engineering and molecular biology. Both N-terminal and C-terminal fusion proteins are contemplated.

Derivation of the pesticidal compounds with long chain hydrocarbons will facilitate passage through the cuticle into the pest body cavity. Therefore, in a further embodiment, the subject invention provides compositions comprising the pesticidal compounds bound to lipids or other carriers.

In addition to the peptide compounds described herein, the subject invention also contemplates that other sterically similar analog compounds may be formulated to mimic the key portions of the peptide structure. Such compounds, which may be termed peptidomimetics, may be used in the same manner as the peptides of the invention and hence are also functional equivalents. The generation of a structural functional equivalent analog may be achieved by the techniques of modeling and chemical design known to those of skill in the art. It will be understood that all such sterically similar analog constructs fall within the scope of the present invention.

The subject invention further contemplates the use of peptide nucleic acids (PNAs) in the practice of the methods of the invention. PNA is a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone. PNA may be utilized in a number of methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (Corey, D.R., 1997, *Trends Biotechnol.* 15:224-229) and is incorporated herein by reference.

neurotransmitters and amino acids (Griffith, J.K. and C.E. Sansom, 1998 In: The Transporter Facts Book, Academic Press, San Diego, pp. 500). All primers are shown in the 5' to 3' direction. The sense primer "S34" (GGIAA(C/T)GTITGG(A/C)G(A/G/C/T)TT(C/T)CC) (SEQ ID NO:3) was based on a GNVWRFP (SEQ ID NO:4) peptide motif, while the antisense primer "S21" (IGC(A/G/T)ATIGCITC(A/G/C/T)GG(A/G)TA) (SEQ ID NO:5) was based on a YP(D/E)AIA (SEQ ID NO:6) peptide motif. Another sense primer "S22" (GGIAA(C/T)GTITGG(G/T)G(A/G/C/T)TT(C/T)CC) (SEQ ID NO:7), a tolerated alternative to S34, was also used in conjunction with S21 antisense primer for initial screening. Another primer set was designed to specifically exclude KAAT1 and other potentially related sequences, including, while amplifying a unique 328 bp segment. In this case, sense primer "S25" (AACACTTGCTGCATCAGTCAAC) (SEQ ID NO:8) and antisense primer "S26" (CTCAAGGAGTTTCGCCCATTG) (SEQ ID NO:9). The S25/S26 set was used for subsequent library phage lysate PCR™ screening steps, and was used with the cloned 943 bp fragment to create a 328 bp digoxigenin (DIG)-labeled (Boehringer-Mannhiem) dsDNA plaque hybridization probe for the isolation of a single clone. The sequence of the full length clone (SEQ ID NO:1) was determined, including the open reading frame plus the 3' and 5' untranslated regions.

Example 2 - DNA Sequence Analysis and Protein Functional Behavior

The complete sequence of the CAATCH1 cDNA clone, including 5′ and 3′ UTRs and the open reading frame encoding the predicted 633 amino acid polypeptide expression product, were obtained for *Manduca sexta* using a midgut cDNA library in LambdaZap phage. The *Manduca* CAATCH1 Genbank accession No. is AF013963 (SEQ ID NO:1).

The CAATCH1 nucleotide coding sequence within the full length clone of 2858 nt (SEQ ID NO:1) contained an open reading frame encoding a predicted unique polypeptide sequence of 633 amino acids (SEQ ID NO:2). The predicted amino acid sequence of CAATCH1 is compared to related members of a subfamily of membrane proteins that includes Na/Cl activated transporters of neurotransmitters (Quick, M. and B.R. Stevens, 2001, *J. Biol. Chem.* 276(36):33413-33418; Feldman *et al*, 2000, *J. Biol. Chem.* 275(32):24518-24526; Griffith, J.K. and C.E. Sansom, 1998, In: The Transporter Facts Book, Academic Press, San Diego, pp. 500) and the *Manduca* KAAT1 leucine transporter (Castagna *et al.*, 1998, *Proc. Natl. Acad. Sci. USA*

95:5395-5400). Based on hydropathy analysis and established membrane protein structure paradigms (Kyte and Doolittle, 1982, *J. Mol. Biol.* 157:105-132), the predicted CAATCH1 protein topology includes 12 putative transmembrane domains, with N- and C- terminal segments residing within the cytosol. Several consensus phosphorylation sites are found within these cytoplasmic segments, and N-linked glycosylation sites exist on the putative extracellular loop between the 3rd and 4th membrane spanning segments. The cytosolic N- and C- terminal regions are relatively rich in proline, acidic, and basic amino residues.

Membrane transporters and ion channels in general can be subcategorized based on thermodynamic properties, substrate selectivities, and reaction mechanism (Gerencser, G.A. and B.R. Stevens, 1994, *J. Exper. Biol.* 196:59-75; Hille, B., 2001, *In*: Ion Channels of Excitable Membranes, 3rd Edition, Sinauer Associates, Inc., Sunderland, Mass, pp 814). CAATCH1 cloned from *Manduca sexta* collectively displayed a unique set of properties (Quick, M. and B.R. Stevens, 2001, *supra*; Feldman *et al.*, 2000, *supra*) that have not been described previously for a given related transporter, including at least the following attributes. The attributes include: (a) the ability to switch particular amino acid substrate selectivities depending on the activator cation Na^+ or K^+, (b) a unique selectivity profile of amino acid-evoked electrical currents, (c) different amino acid substrates directly binding the protein and differentially affecting the conformational states of CAATCH1, apparent lack of chloride ion as a co-activator, (d) Nernstian cation channel behavior independent of amino acid transporter activity, (e) amino acid-modulated ion channel behavior (especially L-methionine binding to CAATCH1 in the presence of Na^+ which perturbs the charge-voltage relation with a high affinity binding constant, affecting transient currents due to CAATCH1-associated charge transfer across the membrane dielectric field), (f) inhibition of current fluxes as the result of binding of methionine to the protein, (g) thermodynamically uncoupled amino acid transport and ion channel behavior, and (h) all these functions behave optimally at an alkaline pH. CAATCH1 defines a new transport system (Quick, M. and B.R. Stevens, 2001, *supra*; Feldman *et al.*, 2000, *supra*; Castagna *et al.*, 1998, *supra*; Christensen, H.N.,1990, Physiol. Rev. 70:43-77; Griffith and Sansom, 1998, *supra*; Kilberg *et al.*, 1993, *Annu. Rev. Nutr.* 13:137-165; Mailliard *et al.*, 1995, *Gastroenterology* 108:888-910; Malandro and Kilberg, 1996, *Annu. Rev. Biochem.* 65:305-336; Stevens, B.R.,1992, "Amino Acid Transport in Intestine" *In*: Mammalian Amino Acid Transport:

TO: MAJ On (note date): 13 JAN 2005 Pat. No.: 6766613
Team Leaders Initials

INFO SUPPLIED BY: _____ 09/991458
OAC/LDRC Initials

SECOND REQUEST (DIFFERENT CORRECITONS), SUPERSEDE OR RECONSIDERATION
(OAC OR LDRC, USE A RED PEN FOR COMPLETING INFO, ON THIS COVER SHEET) (11/2002 cbn)

Team Leader, an Office Automation Clerk may assist you by supplying data from CofC Database (Current & History), PALM, and copies from Intranet, to determine type of request (second request, supersede, and/or reconsideration) and to determine if there were any errors made in decisions and/or publishing are attributable. Team Leader, check appropriate boxes below, key record (if necessary) and forward to JCWS, to order file and assign file to an LIE, to EXPEDITE.

Team Leader, DO NOT ORDER FILE.

MRD (for request attached to this cover sheet): 1/13/05 (Team Leader have LDRC, stamp same MRD on 1050s.)

File Charged to (in PALM): _____ Date Charged to Loc.: __/__/__

Information re most recent record in CofC database(Check Current & History)

MRD: __/__/__ Examiner (LIE's initials): _____

Date Assigned: __/__/__ Turned In: __/__/__

CofC Issued: __/__/__ CofC Denied: __/__/__ Updated: Y / N Date: __/__/__

Patent number listed on C of C listing in OG (circle one) Y / (N)

CofC Issued for this record is attached to patent on Internet (circle one) Y / (N)

New/different correction(s) requested. Check Intranet or with RTIS. (circle one) Y / N

☐ Duplicate (same heading and corrections published/issued CofC on Intranet. (circle one) Y / N
☐ Substitute or corrected request. Locate the original request(check with JCWS and RTIS).
☐ Second Request (another) requesting new/different corrections or additional corrections. TEAM LEADER, DO NOT ORDER FILE. If necessary, call attorney/applicant for assistance in determining if new/different corrections. Team Leader, key new a record on: __/__/__. Place and count with CofCs keyed, same week, determine and note in to upper right hand corner if "P", "R", or "RTC". Mark through any corrections on 1050, that were appropriately published; or JCWS assign to: _____

☐ Reconsideration ☐ Supersede ☐ Special CofC ☐ Erratum ☐ Expedite CofC

Team Leader, determine if a Request for a Corrected CofC (Supersede) or Reconsideration, due to error in decisions or keying, attributable to (check the appropriate box, below):

☐ RTIS Keying Error ☐ LIE: _____ LIE Processing or Decision Error ☐ OFFICE Error in Entry of Document or Ex. Decision ☐ ATTY. 1.323 Consideration or Petition Required

If errors are attributable to LIE, use guidelines for appropriately notifying the LIE and recording errors (make copies supporting that the LIE made error, attach copies to this cover sheet, keeping copies for your records, and forward copies to CBN, at the end of each month).

☐ JW or OL, locate request for CofC published on: __/__/__ and return to: _____
(Circle OAC Initials)

☐ Team Leader keyed record on on: _____ ☐ Post card Printed by Tasneem
(Team Leaders, give all second requests to Tasneem, to print a post card.)

☐ JCWS, order file and assign or reassign to LIE/to: _____/LIE, see your Team Leader for assistance.

Comments/Instructions: _____

☐ SEE REVERSE SIDE, FOR ADDITIONAL COMMENTS/INSTRUCTIONS)

(Revised 12/15/2003 cbn) #4